over Itokawa et al.; of claims 1, 3, 9 and 10 as being obvious over Naruse et al.; of claims 2 and 13 as being obvious over Itokawa et al. or Naruse et al. in view of Horowitz et al.; and of claims 8 and 11 has being obvious over Itokawa et al. and Naruse et al. in view of Vater et al. Applicants traverse these rejections and withdrawal thereof is respectfully requested.

In maintaining the rejections, the Examiner asserts that Applicants' previous arguments were insufficient to overcome the rejections for the following points regarding each reference, which are addressed in turn.

Itokawa et al.:

a) The Examiner asserts that Applicants have argued that the claimed method results in full inactivation of virus; however the claims recite a reduction of viral titre of 10^4. Applicants respectfully note that the recited reduction in viral titre is considered to be synonymous with full inactivation of virus because of the exponential reduction in titre. Attached hereto is a Declaration of inventor GEORG PAULI, which is submitted under 37 C.F.R. §1.132 and wherein the relationship between viral inactivation and reduction of viral titre is discussed.

The factor of 10^4 is calculated from the titre determined in the sample without the inactivating substance (starting titre)

compared to the titre in the sample after treatment (end titre). When at the end of the treatment no infectious virus can be detected any longer in the assay system, the titre is given as the calculated titre (detection limit) and is given by the term $\leq xy$. The reduction factor obtained is therefore limited by the starting titre and the end titre. The lowest reduction factor determined was $\geq 10^4$ (the detection limit) for the inactivation of enveloped viruses with the substance, which means that no virus was detectable after treatment (i.e. full inactivation of the virus). Thus, a reduction of $\geq 10^4$ fold in viral titre is full inactivation of the virus.

b) The Examiner asserts that because the concentrations of Itokawa et al. overlap with those of the claims, the method of Itokawa et al. must inherently result in full virus inactivation. The Examiner has applied an incorrect legal standard regarding a rejection for anticipation by inherency and reconsideration and withdrawal of the rejection is respectfully requested. It is not sufficient to support a rejection based on inherency that one might occasionally happen upon the claimed method by practicing the prior art. The Supreme Court clearly stated in Eibel v Minnesota & Ontario Paper Co., "accidental results, not intended and not appreciated, do not constitute anticipation." Eibel

Processing Co. v. Minnesota & Ontario Paper Co. 261 U.S. 45 (1923). More recently, the Federal Circuit stated in In re Roberton that, "to establish inherency...extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference and it would be so recognized by persons of ordinary skill." In re Roberston 49 USPQ2d 1949 (Fed. Cir. 1999) and in MEHL/Biophile International v. Milgram 52 USPQ2d 1303 (Fed. Cir. 1999) "the mere fact that a certain thing may result from a given set of circumstances is not sufficient...Occasional results are not inherent." MEHL/Biophile International v. Milgram 52 USPQ2d 1303 (Fed. Cir. 1999) Thus, to inherently anticipate the claimed invention, the method of the Itokawa et al. must necessarily fully inactivate viruses. The reference fails to meet the threshold required for establishing a rejection based on inherency.

c) The Examiner asserts that the XTT formazan assay of Itokawa et al. and Weislow et al. is used to measure inactivation of both cell-free HIV-1 and in chronically infected cells. In addition, the Examiner asserts that the *in vitro* assay that measures viral infectivity is equivalent to measuring virus inactivation. Applicants aver that the interpretation of the XTT formazan assay in Itokawa et al. is incorrect. In addition, the

conclusion by the Examiner is a scientific over simplification and "viral infectivity" and "viral inactivation" are two different terms with different accepted meanings in the field of the invention.

Turning again to the Declaration, Dr. PAULI, notes that Itokawa et al. disclose that surfactins (1) and (2) have moderate anti-HIV activities in the XTT formazan assay for HIV-1 cytopathic effects. See page 607, final paragraph preceding "Experimental" section. As in the case of many journal articles, Itokawa et al. does not disclose the details of the XTT formazan assay, but instead references another article where the details are contained. In this instance, Itokawa et al. reference Weislow et al.

The XTT assay method is detailed on pages 579 and 580 of Weislow et al. The assay measures the formation of XTT formazan by infected cells or control cells. Tetrazolium salts (e.g. MTT, XTT, WST-1) are used in cell proliferation/viability assays. The tetrazolium salts are cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1), which belongs to the respiratory chain of the mitochondria and is active only in metabolically active cells. Thus, the Examiner's interpretation of Itokawa et al., i.e. that the reference teaches the direct inactivation of HIV using cell-free viruses in an XTT assay

system, is scientifically impossible because viruses do not have mitochondria or the succinate-tetrazolium reductase enzyme. The Examiner bases her interpretation of Itokawa et al. on the statement in the Abstract that,

> The assay procedure is applicable to the evaluation of drug effects on in vitro infections induced either directly in cultured host cells by cell-free HIV-1 or by coculture with H9 cells chronically infected with HIV-1.

However, the reference in the above statement to cell-free HIV, indicates that the cells assayed in the XTT formazan assay were infected using either cell-free HIV or H9-infected cells. This statement is not an indicative that the direct killing of cell-free HIV was assayed in Itokawa et al. In addition, Weislow et al. state on page 579, right column,

> Uninfected cells or cells that are protected by drugs and have continued to proliferate produce the soluble orange XTT formazan, (fig. 3A) ... Cells not protected by drugs are killed by the virus...

Thus, the XTT formazan assay is used as an indication of the effectiveness of a drug at protecting a cell from a virus and is not an indication of direct virus killing. As indicated above, it is not scientifically possible to measure the direct killing of a virus using the XTT formazan assay because the assay based on mitochondria metabolic activity.

Thus, the bioassay of Itokawa et al. describes the moderate suppression of HIV-1 cytopathic effects on cells, and Itokawa et

al. demonstrates that the drugs being tested act on *virus replication* in cell culture. Itokawa et al. does not disclose that the drugs are acting as virus inactivators or that the drugs destroy the infectivity of products by killing the viruses, as achieved with the present invention.

The Examiner interprets Itokawa et al. (and Naruse et al.) as measuring viral "inactivation", since inhibition of replication would be considered "inactivation." However, as detailed by Dr. PAULI, "viral infectivity" is determined in a viability test, meaning the ability of a treated virus to infect cells in tissue culture (or animals) is tested. "Inactivation of viruses," on the other hand, means the treatment of viruses by a variety of physical and chemical means. (See for both terms: "HIV-1 and other Blood-borne Pathogens", in: Diane O. Fleming and Debra L. Hunt (eds.), Biological Safety: Principles and Practices. Washington: ASM Press, 2000, p. 165). The "inhibition of viral replication" is due to the effect of antiviral agents which interfere with the function of viral proteins, such as AZT, which inhibits the reverse transcriptase of HIV or the protease inhibitors which inhibit the activity of viral protease and thus prevent the virus maturation. See Fundamental Virology, 3rd edition, Eds. Bernard N. Fields, David M. Knipe, Peter M. Howley, Philadelphia/New York: Lippincott-Raven, chapter 26 p. 763 ff.

Naruse et al.:

a) In response to Applicants arguments regarding Naruse et al., the Examiner again asserts that the *in vitro* assay that measures viral infectivity is equivalent to measuring virus inactivation. As discussed above regarding Itokawa et al. the terms "viral infectivity" and "viral inactivation" have two distinct meanings. The discussion regarding these to terms above, is equally applicable to the meaning of the inhibition of "viral infectivity" in Naruse et al.

b) The Examiner asserts that no evidence has been provided that demonstrates that the mechanism of action with the present invention is different than that of Naruse et al. The Examiner is of the position that the fact that Naruse et al. uses the same compounds creates a presumption that the mechanism must be the same. The Declaration of Dr. PAULI demonstrates that the mechanism of action in Naruse et al. must be different from that of the present invention, as demonstrated by the experiments of Naruse et al. those of the specification.

Naruse et al. demonstrate that pumilacidins are inhibitors of herpes simplex type 1. See Abstract. In Naruse et al., antiviral activity was assessed using plaque reduction and dye uptake assays

with HSV-1-Vero cells. See pages 274-275, "*Antiviral Activity.*" In the experiments of Naruse et al., acyclovir was used as a reference compound for antiviral activity. See page 275, lines 5-6. Acyclovir is well-known in the field of virology to be an inhibitor of viral replication. (See for example, the review article: Villarreal EC (2001): "*Current and potential therapies for the treatment of herpesvirus infections*" Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques; Spec No; 185-228.)

Use of acyclovir as the reference compound in the antiviral experiments of Naruse et al., evidences that the mechanism of drug action in the reference is one of inhibition of viral replication, not the direct killing of virus particles, as achieved with the present method.

The mechanism of drug action with the present invention of direct viral killing is evidenced by at least the experiments of Examples 5, 11 and 12 in the specification. In these experiments, the compounds are mixed with cell-free virus particles and the viruses are killed.

Dr. PAULI further states that as one skilled in the art, he would also conclude from the total discussions in Naruse et al. that cyclic lipopeptides are not suitable for the inactivation of viruses in protein-containing biological products. Naruse et al.

discuss in the Abstract, on page 275, Table 5 and *"H^+ and K^+ - ATPase Inhibitory Activity"* that pumilacidins A and B are inhibitors of the enzyme acticity of H^+ and K^+ - ATPase. One skilled in the art would predict from this discussion in Naruse et al. that cyclic lipopeptides could not be used for the inactivation of viruses in protein-containing biological products without negatively affecting the biological activity of the products. In addition, Naruse et al. demonstrate in Table 4, that pumilacidin A and B have ID_{50}'s (concentration for 50% inhibition of cytopathic effects of the non-treated control) that are very similar to the TD_{50}'s, (toxic dose) i.e. a low therapeutic index. This means that the concentrations of pumilacidins required by Naruse et al. for achieving antiviral effects are very close to the toxic concentration. Such a low therapeutic index (difference between the ID_{50} and TD_{50}) would lead one skilled in the art away from using the compounds as antiviral compounds.

Thus, the references relied on by the Examiner fail to teach a method of fully inactivating viruses (as indicated by a reduction in titre of $\geq 10^4$) through the direct killing of the viruses. The present invention is therefore not anticipated by or obvious over the references and withdrawal of the rejections is respectfully requested.

Rejections under 35 U.S.C. §112, second paragraph

The Examiner maintains the rejection of claims 1-9, 13-15, 18 and 19 under 35 U.S.C. §112, second paragraph as being indefinite because no units are included. Applicants respectfully note that a fold reduction in the viral titre is a ratio and thus is the same absolute value regardless of the units used to measure the titre. For example, the Examiner suggests using "ID_{50}/ml" as units. As indicated, the claims recite a ratio or fold reduction in titre. Mathematically, the ratio/fold reduction is calculated as "initial titre X"/"final titre Y". Using the units suggested by the Examiner (or any other units) would result in a calculation of:

$$\frac{X\ ID_{50}/ml}{Y\ ID_{50}/ml}$$

In the calculation of the ratio or fold reduction (in this case with a result of $\geq 10^4$), the units would cancel each other. Thus, contrary to the assertion of the Examiner, no units should be present in the claims and the claims are clear as written.

Rejections under 35 U.S.C. §112, first paragraph for lack of enablement

Claims 1-10, 13-15, 18 and 19 have been rejected under 35 U.S.C. §112, first paragraph for lack of enablement. More specifically, the Examiner asserts that the invention is not

enabled for the preparation of products for *in vivo* administration. The Examiner asserts that the agents used in the present invention are known to be toxic and damaging to blood cells. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The present invention is drawn to a method of inactivating lipid-enveloped viruses in cell-free biological products. The present invention is not drawn to a method of administering products in vivo. The Examiner asserts that "applicants claims encompass any and all biological products...for *in* vivo administration." Applicants again note that present invention does not claim products or therapeutic methods. The present invention claims a method of directly inactivating viruses in a cell-free biological product. Applicants have clearly demonstrated such inactivation with the experiments in the specification. As such, the rejection of the claims is misplaced and not relevant to the claimed invention. Withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. §112, first paragraph - new matter

Claims 1-11, 13-15, 18 and 19 have been rejected under 35 U.S.C. §112, first paragraph as adding new matter with regard to the recitation of "cell-free" biological product. Applicants

traverse this rejection and withdrawal thereof is respectfully requested.

The Examples of the specification, particularly Examples 11 and 12 wherein the inactivation of virus in a cell-free biological product of an albumin solution is disclosed, support the amendment to claim 1 to recite "cell-free." As such, no new matter is added by the term "cell-free" and withdrawal of the rejection is respectfully requested.

If any questions remain regarding the above matters, please contact Applicant's representative, MaryAnne Armstrong, PhD (Reg. No. 40,069), in the Washington metropolitan area at the phone number listed below.

Pursuant to 37 C.F.R. §§1.17 and 1.136(a), Applicants respectfully petition for a two (2) month extension of time for filing a response in connection with the present application. The required fee of $200.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees

required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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